

WHAT IS CLAIMED IS:

1. A method for identifying a cytotoxic T cell epitope comprising the steps in order of:

a) contacting a population of at least two cytotoxic T cells having the same MHC-haplotype restriction with

i) a library of molecules attached to solid phase supports by a releasable linker, wherein each solid phase support is attached to a single species of molecule, and wherein the structure of the molecule can be determined, which library of molecules contains a conserved structural motif corresponding to a structural motif characteristic of peptides that associate with the MHC-haplotype to which the cytotoxic T cells are restricted; and

ii) antigen presentation means, which antigen presentation means correspond to the MHC-haplotype to which the cytotoxic T cells are restricted;

wherein the solid phase supports of the library are in separate fractions;

b) cleaving at least a portion of the releasable linker so as to release at least a portion of the molecule;

c) evaluating whether the cytotoxic T cells recognize a molecule present in one or more of the fractions of the library of molecules;

d) isolating one or more solid phase support(s) from the fractions; and

e) determining the structure of a molecule on a solid phase support isolated from the fraction.

2. The method according to claim 1, wherein the cytotoxic T cells are selected from the group consisting of

a) polyclonal T cells isolated from a site of cytotoxic T cell infiltration from an individual;

b) cells isolated from a site of cytotoxic T cell infiltration from two or more individuals, which two or more individuals share an MHC haplotype;

c) two or more cytotoxic T cell lines; and

d) any combination thereof.

3. The method according to claim 2, wherein the site of cytotoxic T cell infiltration is a tumor.

4. The method according to claim 1, wherein the molecules are peptides.

5. The method according to claim 4, wherein the peptides comprise subunits selected from the group consisting of glycine, L-amino acids, D-amino acids, non-classical amino acids, and peptidomimetics.

6. The method according to claim 1, wherein the solid phase support is selected from the group consisting of polystyrene resin, poly(dimethylacryl)amide-grafted styrene-co-divinylbenzene resin, polyamide resin, polystyrene resin grafted with polyethylene glycol, and polydimethylacrylamide resin.

7. The method according to claim 1, wherein the releasable linker releases upon exposure to an acid, a base, a nucleophile, an electrophile, light, an oxidizing agent, a reducing agent, or an enzyme.

8. The method according to claim 1, wherein the structural motif contained in the library of molecules is selected from the group consisting of **LXXXXXXV** (SEQ ID NO: 1); **RXXXXXX + (SEQ ID NO: 2); X(D, E) XXXXXX(F, K, Y) (SEQ ID NO: 3); RXXXXXXL (SEQ ID NO: 4); X(K, R)XXXXX(L, I) (SEQ ID NO: 5); (M, L)XXXXXXK (SEQ ID NO: 6); EXXXXXX(Y, F) (SEQ ID NO: 7); XPXXXXX(F, H, W, Y) (SEQ ID NO: 8); (L, I)XXXXX(H, K) (SEQ ID NO: 9);** wherein X indicates any amino acid residue, and + indicates a positively charged amino acid residue.

9. The method according to claim 4, wherein a limited number of representative amino acid residues are incorporated in the peptides of the library.

10. The method according to claim 9, wherein positively charged amino acid residues are substituted with an amino acid selected from the group consisting of lysine, arginine, and histidine; negatively charged amino acid residues are substituted with an amino acid selected from the group consisting of aspartic acid and glutamic acid; neutral, polar amino acid residues are substituted with an amino acid selected from the group consisting of asparagine, glutamine, serine, threonine, tyrosine, glycine and cysteine; nonpolar amino acid residues are substituted with an amino acid selected from the group consisting of alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, and methionine.

11. The method according to claim 10, wherein the nonpolar, aromatic amino acid residues are substituted with an amino acid selected from the group consisting of tyrosine, threonine, and tryptophan; and the nonpolar aliphatic amino acid residues are substituted with an amino acid selected from the group consisting of alanine, valine, leucine, isoleucine, proline, and methionine.

12. The method according to claim 1, further comprising a coding molecule attached to each to each solid phase support of the library, which coding molecule defines the structure of the molecule attached to the solid phase support by the releasable linker.

13. The method according to claim 12, wherein the coding molecule is selected from the group consisting of a peptide and an oligonucleotide.

14. The method according to claim 10, wherein the coding molecule is an inert molecular tag that can be decoded by gas-phase chromatography.

15. The method according to claim 1, wherein the antigen presentation means is selected from the group consisting of a purified MHC class I molecule complexed to  $\beta_2$ -microglobulin; an intact antigen presenting cell; and a foster antigen presenting cell.

16. The method according to claim 1, wherein the antigen presentation means is a foster antigen presenting cell.

17. The method according to claim 16, wherein the foster antigen presenting cell lacks antigen processing activity, whereby it expresses MHC molecules free of bound peptides.

18. The method according to claim 17, wherein the foster antigen presenting cell is cell line 174xCEM. T2.

19. The method according to claim 1, wherein the recognition of a molecule present in one or more of the fractions of the library of molecules by the cytotoxic T cells is evaluated by detecting cytotoxic T cell activation.

20. The method according to claim 19, wherein cytotoxic T cell activation is detected by a method selected from the group consisting of  $^3\text{H}$ -thymidine incorporation; metabolic activity detected by conversion of MTT to formazan blue; increased cytokine mRNA expression; increased cytokine protein production; and chromium release by target cells.

21. The method of claim 1, wherein the structure of the molecule is determined by analyzing a portion of the molecule remaining on the solid phase support.

22. The method according to claim 4, wherein a sequence of the peptide is determined by sequencing a portion of the peptide remaining on the solid phase support.

23. The method according to claim 12, wherein the structure of the molecule is determined by analyzing the structure of the coding molecule.

24. The method according to claim 1, wherein the structure of the molecule is determined after isolating more than one candidate solid phase support; repeating steps a) through c), isolating one solid phase support in step c), and determining the structure of a molecule on the solid phase support isolated in step c).

25. The method according to claim 9, further comprising the steps in order of:

- contacting the population of at least two cytotoxic T cells having the same MHC-haplotype restriction with

i) a library of molecules attached to solid phase supports by a releasable linker, wherein each solid phase support is attached to a single species of molecule, and wherein the structure of the molecule can be determined, which library of molecules contains a conserved structural motif corresponding to a structural motif characteristic of peptides that associate with the MHC-haplotype to which the cytotoxic T cells are restricted, and wherein every amino acid corresponding to the representative residue is utilized at the position identified for the corresponding representative residue; and

ii) antigen presentation means, which antigen presentation means correspond to the MHC-haplotype to which the cytotoxic T cells are restricted;

wherein the solid phase supports of the library are in separate fractions;

b) cleaving at least a portion of the releasable linker so as to release at least a portion of the molecule;

c) evaluating whether the cytotoxic T cells recognize a molecule present in one or more of the fractions of the library of molecules;

d) isolating one or more solid phase support(s) from the fractions; and

e) determining the structure of a molecule on a solid phase support isolated from the fraction.

26. A method for identifying a high affinity cytotoxic T cell epitope comprising:

a) contacting a population of cytotoxic T cells having an MHC-haplotype restriction with

i) a library of molecules attached to solid phase supports by a releasable linker, wherein each solid phase support is attached to a single species of molecule, and wherein the structure of the molecule can be determined, which library of molecules contains a conserved structural motif corresponding to a structural motif characteristic of peptides that associate with the MHC-haplotype to which the cytotoxic T cells are restricted, and wherein every amino acid corresponding to a representative residue determined according to the method of

claim 9 is utilized at the position identified for the corresponding representative residue; and

ii) antigen presentation means, which antigen presentation means correspond to the MHC-haplotype to which the cytotoxic T cells are restricted;

wherein the solid phase supports of the library are in separate fractions;

b) cleaving at least a portion of the releasable linker so as to release at least a portion of the molecule;

c) evaluating whether the cytotoxic T cells recognize a molecule present in one or more of the fractions of the library of molecules;

d) isolating one or more solid phase support(s) from the fractions; and

e) determining the structure of a molecule on a solid phase support isolated from the fraction.

27. A method of identifying a protein antigen comprising:

a) identifying the cytotoxic T cell epitope of the protein according to the method of claim 25;

b) comparing a sequence of the T cell epitope identified in step (a) with known sequences of proteins; and

c) determining a protein having a sequence corresponding to the sequence of the T cell epitope.

28. A method of identifying a protein antigen comprising:

a) identifying the cytotoxic T cell epitope of the protein according to the method of claim 26;

b) comparing a sequence of the T cell epitope identified in step (a) with known sequences of proteins; and

c) determining a protein having a sequence corresponding to the sequence of the T cell epitope.